New Vessel Design for Rapid, Continuous Fermentation

U.S. Department of Agriculture
Science and Education Administration
Advances in Agricultural Technology • AAT-W-10/February 1980

ABSTRACT

Tests were made of a new vessel design for the continuous fermentation of liquids. Grape juice and nutrient glucose solutions were fermented at a pumping rate of one vessel volume per 4 hours continuously for long periods without difficulty. No loss of yeast activity or contamination problems were encountered during a continuous 52-day run.

This publication is available from the Western Regional Research Center, 800 Buchanan St., Albany, Calif. 94710.

International Standard Serial Number (ISSN) 0193-3736

Science and Education Administration, Advances in Agricultural Technology, Western Series, No. 10, February 1980

Published by Agricultural Research (Western Region), Science and Education Administration, U.S. Department of Agriculture, Oakland, Calif. 94612

2445

NEW VESSEL DESIGN FOR RAPID, CONTINUOUS FERMENTATION

By Emil Wick[1]

INTRODUCTION

The suitability of a particular vessel for continuous fermentation depends greatly upon how its size and shape influence the mixing patterns produced by gas bubbles evolving during fermentation. The objectives of good design are as follows:

1. Inflowing liquid is held in intimate contact with active yeast long enough to permit complete fermentation.

2. The fermented liquid is then separated from the yeast and leaves the vessel while yeast is retained.

3. High sugar concentrations are maintained within the vessel through which the yeast can occasionally pass.

It seemed that these objectives of good design could be met by slanting the bottom of a rectangular tank. Fermentable liquid is pumped in near the bottom while product overflows at the shallow end.

Gas bubbles streaming upwards from the sediment at the bottom of the vessel cause the liquid mass to rotate by the gas-lift principle. Yeast cells tend to circulate with the large mass of rotating liquid rather than to leave with product, which overflows at the shallow edge. Furthermore, the yeast repeatedly pass through fresh, high-sugar liquid, entering the deep part of the vessel.

Just how successful this simple design principle could be in maximizing fermentation rates was the subject of this work. Two slant-bottom fermentor models were built similar in shape but different in size. They were tested in the continuous fermentation of grape juice and nutrient glucose solutions. Details of vessel construction, fermentation tests, and results are reported here.

---

[1]Research food technologist, Food Engineering Applications, Western Regional Research Center, Science and Education Administration, U.S. Department of Agriculture, Albany, Calif. 94710.

MATERIALS AND METHODS

The fermentor models (fig. 1) were constructed as triangular wafers rather than full width in order to minimize the volume of juice required for testing. They were made of clear acrylic plastic for good visibility. The vessels stood upright upon supports with braces to prevent the flat sides from bulging when full. In the smaller vessel, with which this study started, the liquid occupied a right triangular space 122 cm high, 130 cm long, and 2.5 cm wide. Headspace height was 15 cm. Vessel capacity was 23 liters. Both gas and wine exited the covered vessel at the shallow end through a 0.6-cm pipe nipple and tee.

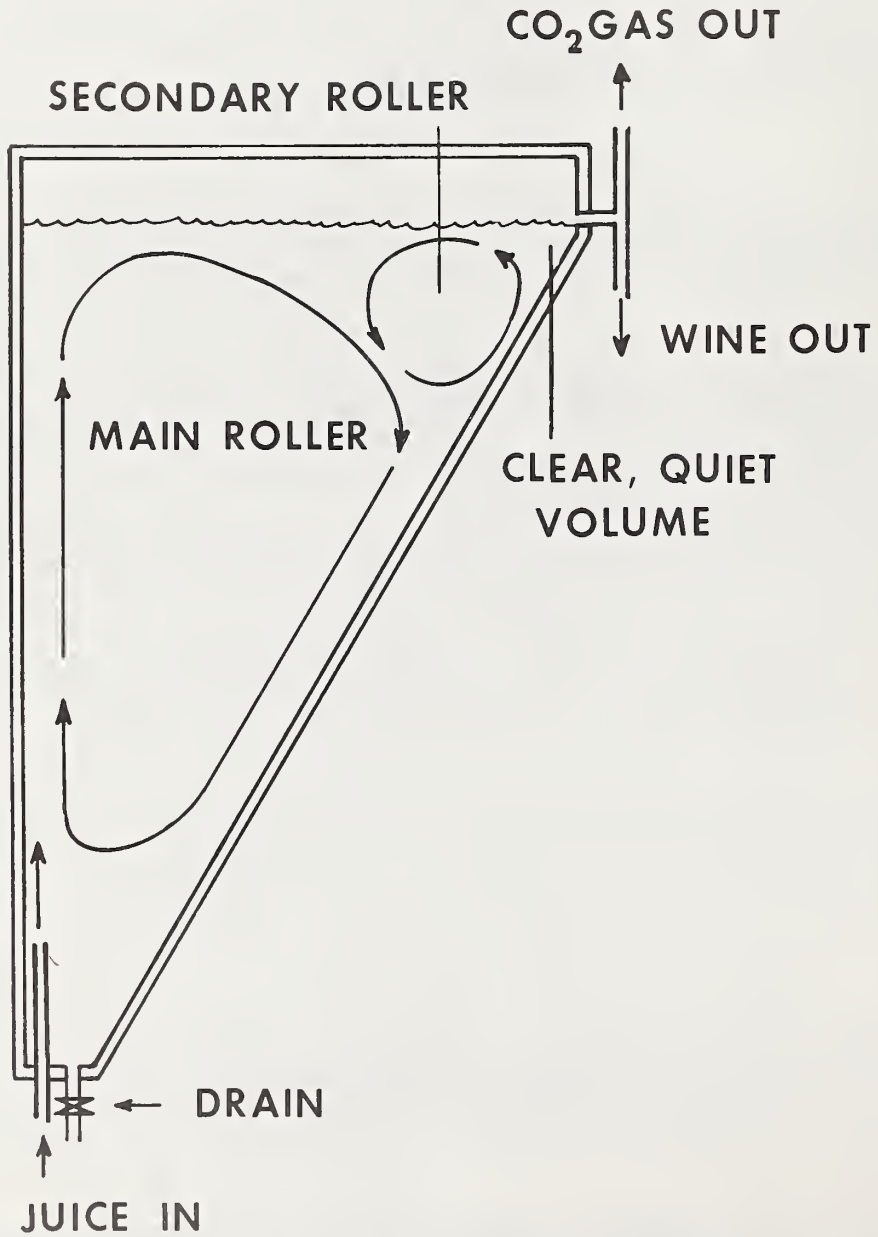

Figure 1.--Profile view of the experimental fermentor showing the flow patterns maintained during fermentation.

In the larger vessel, the liquid occupied a right triangular space 180 cm high, 120 cm long, and 5 cm wide. The bottom apex of the vessel was truncated, providing a flat bottom 10 cm long. Vessel capacity was 65.5 liters. The exit nipple and tee were made of 1.27-cm pipe. In both vessels, the influent juice pipe extended upwards from the bottom of the vessel to about one-fifth the liquid depth.

Three kinds of substrate were fermented--white grape juice reconstituted from concentrate and two blends of this with a glucose:nutrient salts solution in the proportions 1:1 and 1:4. The glucose:nutrient salts formula was as follows:

	Grams/liter
Glucose (commercial cerelose).....	180
Ammonium monobasic phosphate.....	6.0
or urea.....	3.0
Sodium citrate.....	1.0
Yeast extract, dried.....	1.0
Magnesium sulphate.....	.25
Potassium monobasic phosphate.....	.20
Ferrous ammonium sulphate.....	Trace
Zinc sulphate.....	Trace
Cupric sulphate.....	Trace

Initial SO_2 was 125 p/m ± 5 pm. The yeast source was a commercial, dried preparation of Montrachet Wine Yeast. Fermentation temperatures averaged 26° ± 2°C.

The general procedure for making the fermentation tests was to begin with the vessels about one-tenth full of an actively fermenting juice, then to pump fresh juice steadily into the vessel from a refrigerated reservoir. The juice was held in the reservoir at 1° to 2°C to prevent prefermentation. Pumping was continued for at least 2 days after the effluent sugar concentration became minimal. During the entire fermentation time, the effluent was sampled two to three times throughout the day and analyzed for sugar and alcohol. CO_2 volume rates were measured at the effluent gas pipe by water displacement.

After steady-state had been established at one pumping rate, a step increase in the pumping rate was made and continued until another steady-state was reached. In this way, nominal juice residence time (i.e., fermenting volume divided by juice volume rate) was reduced in steps until a limit was reached, which was signalled by a jump in the unfermented sugar content of the effluent wine. Continuous fermentation was maintained for 11 days in the smaller vessel,

then stopped when it developed leaks. Fermentation was maintained for 52 days in the larger vessel without difficulties.

RESULTS AND DISCUSSION

In both vessels, percent of sugar fermented during steady-state remained constant as the juice pumping rate was increased (fig. 2, table 1). Each step increase in the pumping rates was met by a corresponding increase in fermentation rate, usually within 48 hours, so that the effluent sugar concentration, which rose temporarily when the pumping rate was increased, returned to its previous level.

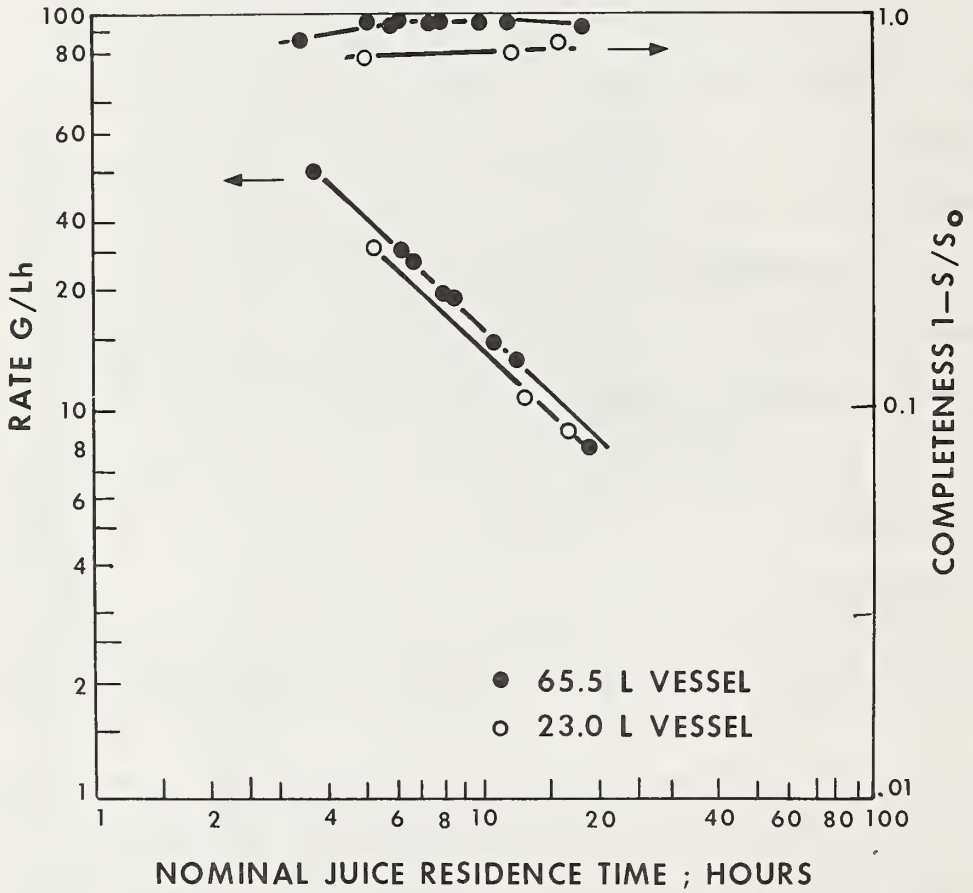

Figure 2.--The effect of juice residence time upon the rate and completeness of fermentation.

A limit was reached at a juice pumping rate corresponding to a nominal juice residence time of 3.6 hours. At this point, excessive yeast washout started. Large clumps of yeast were intermittently pushed out of the vessel, visibly diminishing the amount present. Over most of the juice pumping rates tried, the effluent wine was remarkably clear of yeast--usually no more than about 10^6 cells/cm^3.

At the conclusion of the 52-day run, the fermentor contents were drained into a container, mixed well, then sampled for yeast volume determination. The centrifuged yeast volume was 15 percent of the total volume, which corresponded to a cell count of 1.7×10^9 cells/cm^3. By comparison, batch wine fermentations of this substrate achieved cell counts of 1.4×10^8 cells/cm^3. The results show that this fermentor design allows cells to accumulate to densities much greater than those achievable in batch fermentation, thus greatly decreasing fermentation time in comparison with batch methods.

Juice composition made no difference in the fermentations. The important thing was that cell retention was effective enough to allow cell density to increase by growth up to the limit permitted by the juice (i.e., nutrient) supply rate. Under these conditions, growth rate differences due to substrate can only affect the rate of response of the cell population to changes in the juice pumping rate. Even with slow growth, enough cells will eventually accumulate to ferment all of the juice.

The gross mixing pattern was the same in both vessels (fig. 1). A single, large, rotating liquid mass occupied 79 percent of the fermentation volume in the smaller vessel and 67 percent in the larger. In both vessels, a smaller liquid mass nearer the effluent end, rotated counterclockwise to the main liquid mass. Just at the effluent end, there was a small volume of clear, stagnant wine. Quite remarkably, yeast particles from the main body of fermenting juice seldom entered this clear zone. Only during yeast washout did yeast particles cross over. These flow patterns were very distinct during fast juice pumping but were less so at slower rates.

A dense mass of yeast accumulated in the deepest part of each vessel but did not settle into a compact sediment. The mass remained mobile. Periodically, CO_2 gas bubbles, which had accumulated within the yeast mass, burst upwards, dispersing the yeast throughout the vessel. Stagnant volumes appeared to represent less than 10 percent of the total vessel volume.

The fermentation rate data prove there was no reduction in the fermentative vigor of the yeast during the entire period of the fermentation. Furthermore, the normal odor of the effluent wine gave evidence that the fermentations were free of infecting organisms, e.g., *Acetobacter*, even though no particular effort towards cleanliness was made other than rinsing equipment with tapwater. Complete reliance was placed upon sulfiting the juice to 125 p/m SO_2 at the start. Thus, it appears possible to maintain a continuous wine fermentation rate of one vessel volume per 4 hours indefinitely without difficulty.

The maximum percent sugar fermented at steady-state that could be attained with each vessel appeared to be a function of the size of the fluidized yeast mass. Fermentation was more nearly complete in the larger vessel and so, too, was the size of the fluidized yeast mass. This suggests that complete fermentation, i.e., 99 percent or better, should be attained in vessels of commercial size.

Percent sugar fermented dropped to 92 percent at the slowest juice pumping rate tried with the larger vessel because gas evolution then became too slow to fluidize the yeast mass sufficiently. The yeast occupied a noticeably shallower layer, which had the effect of making the vessel smaller.

Scale up of this design, for commercial use, should present no difficulties. Flow patterns in full-width prototypes should be similar to and equally effective as in the wafer models because the dominating influence in both is the very large difference in bubble pumping power across the vessel created by the extremely slanted bottom. Only simple refinements of the design should be necessary to prevent an increase of stagnant volume as vessel width is increased. For example, it may be better to introduce the juice through a manifold into a full-width vessel rather than at a single point as was done in the wafer models. A U.S. Patent Application (No. 900,371, "Fermentation of Liquids") has been made for this design.

SELECTED REFERENCES

(1) Wick, E., K. Popper, and R. P. Graham.
1974. Performance characteristics of yeast-alcohol fermentors with no mechanical stirring. Biotechnology and Bioengineering 16:1611-1631.

(2) Wick, E., and K. Popper.
1977. Continuous fermentation in slant tubes. Biotechnology and Bioengineering 19:235-246.